# PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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# PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 24.02.2004 |
| Applicant's or agent's file reference<br>P.ULB.71/WO | **IMPORTANT NOTIFICATION** |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/BE 03/00045 | 19.03.2003 | 19.03.2002 |

Applicant
UNIVERSITE LIBRE DE BRUXELLES et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

| Name and mailing address of the international preliminary examining authority: | Authorized Officer |
|---|---|
| European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Büchler, S<br>Tel. +49 89 2399-8090 |

# PATENT COOPERATION TREATY

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# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| P.ULB.71/WO | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/BE 03/00045 | 19.03.2003 | 19.03.2002 |
| International Patent Classification (IPC) or both national classification and IPC | | |
| C12N15/82 | | |
| Applicant | | |
| UNIVERSITE LIBRE DE BRUXELLES et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 10.10.2003 | 24.02.2004 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized Officer<br>Wimmer, G<br>Telephone No. +49 89 2399-7347 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/BE 03/00045

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, Pages**

1-9 as originally filed

**Claims, Numbers**

1-14 filed with telefax on 12.02.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-14 (partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☒ the claims, or said claims Nos. 1-14 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-14 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-14 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 1-14 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

**Re Item III**

**Non-establishment of opinion.**

1) Claims as amended before the International Preliminary Examination Authority refer to constructs containing a sequence encoding a toxic gene, and a sequence encoding an antidote molecule. This is, however, a broader definition than that of the application as originally filed, wherein it is stated that the construct will have a specific configuration (LB-ANTITOX-TOX-selectable marker A-RB), or that the antidote is introduced in an episomal DNA.
For the purpose of this preliminary examination, claims were regarded to be limited to such subject-matter as originally disclosed.

2) Claim 9 refers to the integration of a genetic sequence "which is the target of the toxic molecule" (so that the target of the toxin is DNA). However, the description rather refers to sequences which *encode* the target of the toxic molecule (so that the target of the toxin is a protein). Claim 9, and all claims referring thereto, therefore lack clarity and/or enablement.
For the purpose of the present preliminary examination, claims were regarded to refer to sequences which encode the target of the toxic protein.

**Re Item V**

**Reasoned statement under Art. 35(2) PCT with regard to novelty, inventive step or industrial applicability.**

3) Reference is made to the following documents (the document numbering corresponds to their order of citation in the international search report):

D1: DE 100 38 573 A (MPB COLOGNE GMBH MOLECULAR PLA) 21 February 2002 (2002-02-21)

D2: WO 97 13401 A (LEE JANG YONG ;HODGES THOMAS K (US); HUQ ENAMUL (US); LYZNIK LESZE) 17 April 1997 (1997-04-17)

D3: GABANT P ET AL: 'USE OF POISON/ANTIDOTE SYSTEMS FOR SELECTIVE CLONING' PLASMID, NEW YORK,NY, US, vol. 45, no. 2, 19 September 2000 (2000-09-19), pages 160-161, XP001077797 ISSN: 0147-619X

Novelty under Art. 33(2) PCT.

4) Various documents of the prior art describe systems of host cells, such as plant cells, which have been modified to contain a gene encoding a toxin under the control of an inducible promoter (D1, D2). Furthermore, e.g. D1 envisions in preferred embodiments the toxin containing construct to be integrated into the genome; a Ti-plasmid to be used for transformation; the integration of the sequence into a plastid or mitochondria genome; and the replacement of the sequence to be excised with a desired DNA sequence.

However, these documents do not describe the simultaneous introduction of an antidote gene. Subject-matter of present claims 1-14 is therefore considered to be novel.

Inventive Step under Art. 33(3) PCT.

5) Document D1 describes a system for the excision of a specific sequence by selection for the absence of a toxin encoding DNA sequence, and also describes replacement of this toxin encoding sequence with a desired DNA sequence through homologous flanking sequences. D1 thereby provides a method for site-specific integration of a desired DNA sequence through replacement of a toxin encoding gene which is under the control of an inducible promoter.

However, the prior art does not clearly lead the skilled person to include an antidote gene in such a toxin excision/replacement method, to antagonise "leaky" expression of a toxin from an inducible promoter. An inventive step may therefore be acknowledged for subject-matter of claims 1-14, keeping in mind the limitation of examined subject-matter as discussed above under sections III.1 and III.2.

CLAIMS

1. A recombinant eucaryote cell or organism with the provisio that it is not a human germ cell line, a human zygote, a human embryo or a human individual, said cell or organism having incorporated in its genome

(i) a genetic construct made of at least one nucleotide sequence and possibly a selectable marker, said sequence encoding a toxic gene (TOX) under the control of an inducible promoter/operator genetic sequence and

(ii) a genetic sequence encoding an antidote molecule to said toxic molecule with the condition that the sequence encoding the antidote molecule is not present natively in said cell or organism.

2. The recombinant eucaryote cell or organism according to the claim 1, wherein the genetic sequence encoding the antidote molecule is under the control of an inducible promoter/operator genetic sequence.

3. The recombinant eucaryote cell or organism according to claim 1 or 2, wherein the genetic sequence encoding a toxic molecule is a genetic sequence encoding a poison protein, selected from the poison/antidote group.

4. The recombinant eucaryote cell or organism according to the claim 3, wherein the genetic sequence encoding the toxic molecule is a genetic sequence encoding a poison protein selected from the group consisting of CcdB, ParE, RelE, Kid, Doc, MazF, Hok proteins.

5. The recombinant eucaryote cell or organism according to claim 1 to 4, which is a plant or a plant cell.

6. The recombinant eucaryote cell or organism according to claim 1 to 4, which is an animal cell

or an animal organism, preferably a mammalian cell or a mammalian organism.

7. The recombinant eucaryote cell according to claim 1 to 4, which is a yeast cell.

8. The recombinant eucaryote cell or organism according to anyone of the preceding claims, wherein the inducible promoter/operator genetic sequence is induced by a non-toxic compound, preferably a exogenous compound or a compound that is synthesized by the eucaryotic cell or organism itself, preferably at a specific stage of its development or in a specific tissue.

9. The recombinant eucaryote cell or organism according to anyone of the preceding claims, which further comprises integrated into the genome, a genetic sequence which is the target of the toxic molecule.

10. The recombinant eucaryote cell or organism according to anyone of the preceding claims, wherein the genetic construct is integrated into the genome of specific cell compartments, such as chloroplasts or mitochondria.

11. The recombinant eucaryote cell or organism according to anyone of the preceding claims, wherein the selectable marker is bordered by two different or identical toxic genes.

12. A production and selection method of a genetically modified eucaryote cell or organism having integrated into their genome foreigner (exogenous) DNA fragment(s) which comprises the steps of (i) providing the recombinant eucaryote cell or organism according to any one of the preceding claims 1 to 11 with the genetic construct carrying the toxic gene integrated therein, (ii) providing a construct carrying said foreigner DNA fragment; (iii) obtaining the integration, in the genome of the recombinant eucaryote cell, of said foreigner (exogenous) DNA

fragment(s) at the insertion site where the genetic construct is integrated; (iv) selecting the genetically modified eucaryote cell or organism having integrated said foreigner (exogenous) DNA fragment(s) under condition allowing the expression of the toxic molecule in said cells or organisms; and (v) recovering said genetically modified eucaryote cells or organisms which do not express said toxic molecule following the integration of the foreigner (exogenous) DNA fragment(s).

13. The production and selection method according to claim 12, wherein said foreigner (exogenous) DNA fragment(s) are integrated into the genome of the recombinant eucaryote cell or organism preferably by homologous recombination between the sequence of said foreigner (exogenous) DNA fragment(s) and the sequence of the genetic construct integrated into the genome of the recombinant eucaryote cell or organism.

14. The method according to claim 12 or 13 wherein said eucaryote cell or organism is a plant or a plant cell transfected by a Ti-plasmid incorporating the toxic gene and being preferably present in *Agrobacterium tumefaciens* and wherein a complete transgenic plant is possibly obtained from the recovered genetically modified plant cell.